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Biodegradation of weathered crude oil in seawater with frazil ice

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ABSTRACT

As ice extent in the Arctic is declining, oil and gas activities will increase, with higher risk of oil spills to the marine environment. To determine biotransformation of dispersed weathered oil in newly formed ice, oil dispersions (2–3 ppm) were incubated in a mixture of natural seawater and frazil ice for 125 days at −2 °C. Dispersed oil in seawater without frazil ice were included in the experimental setup. Presence or absence of frazil ice was a strong driver for microbial community structures and affected the rate of oil degradation. *n*-alkanes were degraded faster in the presence of frazil ice, the opposite was the case for naphthalenes and 2–3 ring PAHs. No degradation of 4–6 ring PAHs was observed in any of the treatments. The total petroleum oil was not degraded to any significant degree, suggesting that oil will freeze into the ice matrix and persist throughout the icy season.

1. Introduction

With the warming of the Arctic, sea ice extent and coverage is declining; a trend that is likely to give a seasonal ice free Arctic within this century (Serreze and Meier, 2019). As a consequence, previously closed polar navigation routes are becoming accessible for shipping, and technical challenges related to petroleum exploration are reduced. It has been estimated that the unproduced or undiscovered amount of oil north of the Arctic Circle may be as high as 90 billion barrels, most of it in offshore areas (Bird et al., 2008). As activities increase, so does the risk of accidental oil spills from oil installations and ships. Dependent on prevailing wind and current conditions, discharged oil may drift into ice covered areas with variable degree of ice coverage and type (Wadhams et al., 1988; Quillfeldt et al., 2018), an environment where oil spill mitigation can be extremely challenging.

Oil spilled into the marine environment will undergo physical, chemical and biological weathering that alters the oil properties over time. These processes include: evaporation, emulsification, natural dispersion, dissolution of water-soluble compounds, photo-oxidation, sedimentation and biodegradation (Daling et al., 2014; Lee et al., 2015). Typically, weathering results in increased oil viscosity, as well as increased density and pour-point (Lee et al., 2015). Thus, weathering might complicate mechanical oil spill clean-up, as well as biodegradation, because of reduced dispersibility (Brakstad et al., 2014). Efficient use of dispersants can facilitate natural degradation processes in the water column, as smaller oil droplets will degrade faster than larger ones under the same environmental conditions (Brakstad et al., 2015a). Reduced oil droplet size results in a higher surface-to-volume ratio and increases bioavailability of hydrocarbons. Oil-degrading microorganisms will therefore have a larger surface area to attach to. Oil biodegradation is strongly dependent on environmental factors not only affecting the indigenous microbial communities, but also the oil properties and behaviour. Cold temperatures affect oil bioavailability by altering solubility, viscosity and fluidity of the oil, making access to hydrocarbons more difficult for the microorganisms. This will subsequently result in slower degradation rates (Brandvik and Faksness, 2009; Lofthus et al., 2018).

During an oil spill, the microbial communities will change dynamically in response to changes in the oil properties and composition (Ribicic et al., 2018a; Vergeynst et al., 2018). A few hydrocarbonoclastic species typically dominate in the initial phase, degrading easily accessible *n*-alkanes and volatile compounds, and these will be succeeded by a more diverse consortium of genera degrading polycyclic aromatic hydrocarbons (PAHs) and increasingly more complex structures (Lofthus et al., 2018; Head et al., 2006; Ribicic et al., 2018c; Dubinsky et al., 2013). Most oil-degrading bacteria belong to the classes Alpha- and Gammaproteobacteria. Typically, organisms of the families *Alcanivoraceae* (*Alcanivorax*) and *Oceanospirillaceae* (*Oleibacter*, *Oleispira*) are detected early in an oil spill event, followed by members of families such as *Piscirickettsiaceae* (*Cycloclasticus*), *Colwelliaceae*

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(*Colwellia*) and *Rhodobacteraceae* (Dubinsky et al., 2013; Ribicic et al., 2018b). Hydrocarbon degraders have previously been detected in both Arctic and Antarctic sea ice contaminated with fresh crude oil (Deppe et al., 2005; Gerdes et al., 2005; Delille et al., 1997). Oil degrading microbes may also inhabit the brine channels of the ice interior. Most of these are Gammaproteobacteria, including known oil degraders of the genera *Colwellia, Marinomonas* and *Glaciecola* (Brakstad et al., 2008; Boccadoro et al., 2018).

The marginal ice zone (MIZ), the transition zone where pack ice meets the open ocean, is an area that is characterized by seasonally high primary production and biological activity (Quillfeldt et al., 2018). The relatively short, but intense spring bloom drives migration, foraging and reproduction of zooplankton, which are key organisms for energy transfer from primary producers to higher tropic levels, emphasising the importance of the sea ice environment (Arrigo et al., 2012; Barber et al., 2015; Daase et al., 2013). From an oil spill response point of view, oil drifting into MIZ might be regarded as the worst possible scenario, as the uncertainty is high regarding the effect of a potential oil spill in this area (Lee et al., 2015). Difficulties in performing traditional mechanical clean-up would result in a lengthening of the contamination and exposure times. (Lewis and Prince, 2018). Oil might become incorporated into ice, either newly formed ice or more mature ice forms. A possible scenario is that dispersed oil in the upper part of the water column are scavenged by newly formed ice crystals, known as frazil ice, together with algae and bacteria (Garrison et al., 1983). Frazil ice is formed by super cooling of the water column under turbulent conditions and rise to the surface freezing into what will eventually become solid first year ice (Petrich and Eicken, 2010).

The objective of this study was therefore to investigate biodegradation of oil spilled in cold seawater, which was treated with chemical dispersants before coming in contact with newly formed frazil ice. While previous biodegradation studies have focused on releases of fresh oil in and under solid ice (Brakstad et al., 2008; Garneau et al., 2016; Boccadoro et al., 2018; Vergeynst et al., 2019; Gerdes et al., 2005), little is known about biodegradation of dispersed weathered oil freezing into growing ice. We therefore performed a biotransformation experiment of chemically dispersed weathered oil in the presence of frazil ice and compared it with samples that did not contain any ice. Studying degradation of total hydrocarbons, target *n*-alkanes and PAHs, as well as the successions of the microbial communities over time.

2. Materials and methods

2.1. Seawater and frazil ice

Seawater was collected from a depth of 80 m in a Norwegian fjord (Trondheimsfjorden; 63°26′N, 10°23′E) and transported to the SINTEF laboratories through a continuous flow pipeline system and filtered through a sand filter to remove coarse particles. Microbial communities at the pipeline inlet (80 m depth) and after collection in the laboratory (after sand filter) have approximately the same composition (see Fig. S1, Supplementary Information). The inlet of seawater is considered not to be influenced by seasonal variations, being located below the thermocline. Frazil ice was made from the collected seawater, in a stirring tank at a temperature of −20 °C overnight, as previously reported (Top et al., 1988). Ice was collected with a sieve and stored in a clean 60 L container.

2.2. Oil droplet dispersions

An evaporated residue of the naphthenic North Sea crude oil Troll B was used in this experiment. The oil was heated to a temperature of 250 °C (Troll B 250+), which corresponds to the weathering after 2–5 days at sea (Daling et al., 1990). The density and pour point of the weathered oil were 0.922 g/ml and −3 °C, respectively (the density and pour point of the fresh oil were 0.894 g/ml and < −36 °C). The weathered oil had lost 27% of its original volume due to evaporation. The Troll B 250+ oil was pre-mixed with a dispersant (Corexit 9500; Nalco Environmental Solutions LLC, Suger Land, Tx, USA) at a dispersant-to-oil ratio of 1:100. Stock dispersions of oil were prepared in an oil droplet generator, as previously described Nordtug et al. (2011). This method allows generation of oil dispersions with a uniform droplet size distribution and controlled concentration, by adjusting the water flow and oil flow, respectively. Stock dispersions were prepared with a nominal oil droplet concentration of 200 mg/L, and a median droplet size of 10–20 μm. Oil droplet size and concentrations were determined by Coulter Counter analysis.

2.3. Biodegradation experiment and sampling

To investigate the biodegradation of oil in frazil ice, an experimental model system based on glass bottles mounted on rotating carousels was prepared. The experiment was conducted with four treatments: samples with seawater, frazil ice and oil (FI + oil), controls with seawater and oil (without frazil ice; SW + oil), biotic controls with frazil ice and seawater (without oil; FI), and abiotic controls with seawater and oil (without frazile ice; abioticSW + oil).

Oil dispersions were prepared by diluting the stock dispersions to a nominal concentration of 2–3 mg/L, in 2 L flasks (Schott), completely filled (no headspace) with equal volumes of frazil ice and seawater in (FI + oil) or just seawater (SW + oil). Abiotic controls (aboiticSW + oil) were prepared with sterile-filtered [0.22 μm] seawater supplemented with $HgCl_2$ [100 mg/L], with the same nominal concentrations of oil as the FI + oil and SW + oil samples. Biotic controls without oil (FI) contained frazil ice and seawater in the same manner as the FI + oil samples. An overview of all treatments is shown in Table 1. All flasks were mounted on a carousel with slow continuous rotation of 0.75 rpm as previously described Brakstad et al. (2015a) and incubated in the dark at −2 °C in a temperature-controlled room for up to 125 days. All flasks were baked, washed and autoclaved prior to sample preparation to minimize glass wall adherence of oil (Brakstad et al., 2015a). Growth or melting of the frazil ice phase was visually inspected frequently, and the temperature was adjusted accordingly, to maintain a constant volume of frazil ice and prevent ice controls from completely freezing. Flasks were sacrificed for analyses at the start of the experiment (day 0, after 15–20 min mixing), and after incubation for 14, 35, 55, 76, 88, and

Table 1
Treatments.

Treatment	Label		Volume (L)			Oil concentration (ppm)	Oil	Replicates
		Fractions	Total	Ice	Sea water			
Frazil Ice sample	FI + oil	FI + oil/SW FI + oil/ice	2.3	1.15	~1.15	2.5	Troll B 250+	3
Sea water control	SW + Oil		2.3	–	~2.3	2.5	Troll B 250+	2
Abiotic control	abioticSW + Oil		2.3	–	~2.3	2.5	Troll B 250+	1
Blank control	FI	FI/SW FI/ice	2.3	1.15	~1.15	0	–	1

125 days. FI + oil samples were collected as triplicate samples at each sampling time, while SW + oil was sampled as duplicates. Biotic (FI) and abiotic controls (aboiticSW + oil) were sampled as single samples. Samples containing frazil ice (FI + oil and FI) were separated into a seawater and an ice fraction (FI + oil/SW and FI + oil/ice; FI/SW and FI/ice) upon sampling. Frazil ice was allowed to melt at 4 °C overnight before further analysis. Aliquots for analysis of dissolved oxygen (DO; 50 ml), salinity (1 ml), most probable number (MPN; 1 ml) and epifluorescence microscopy (10 ml) were collected and analyses performed on the day of sampling. Aliquots for microbial community analyses (500 ml volumes) were filtered through 47 mm polyethersulphone membranes (pore size 0.22 μm; Millipore). The filters were folded and stored in sterile tubes (−18 °C) until DNA extraction. The remaining volume of each flask was acidified (pH < 2) and stored at 4 °C until extraction of hydrocarbons for chemical analyses.

2.4. Chemical analysis and calculation of biodegradation rates

Acidified samples were solvent-solvent extracted with dichloromethane (DCM) for measurements of semivolatile organic compounds (SVOC). A gas chromatograph coupled to a flame ionization detector (GC-FID; Agilent 6890 N with 30 mDB1 column; Agilent Technologies, Inc. CA, USA) was used for quantification of C_{10}-C_{36} total petroleum hydrocarbons (TPH). *o*-Terphenyl (100 μg/ml) was used as surrogate internal standard (SIS), and 5a-androstane (100 μg/ml) as recovery internal standard (RIS). Targeted analytes were quantified in a gas chromatograph coupled to a mass spectrometer (GC–MS; Agilent 6890 plus GC coupled with an Agilent 5973 MSD detector, operated in Selected Ion Monitoring [SIM] mode; Agilent Technologies, Inc. CA, USA). GC–MS analyses included *n*C10-*n*C36 alkanes, 2- to 6-ring polycyclic aromatic hydrocarbons (PAH, $n = 43$; listed in Table S1, Supplementary Information) and 17α(H),21β(H)-hopane (30ab hopane), as previously described (Brakstad et al., 2014, 2015a). Deuterated SIS-PAH (naphthalene, phenanthrene, chrysene, perylene; 5–25 μg/ml) and RIS-PAH (acenaphthene, fluorene; 10 μg/ml) were included for calculation of analytical recovery. The response values for individual target analytes were determined, with a signal-to-noise ratio of 10, and a lower limit of detection (LOD) of 0.01 μg/L was defined for individual oil compounds. Experimental blanks (deionized water) and a QA-oil spike (a standard fresh paraffinic oil) were included in analyses of all test batches for GC-FID and GC–MS analyses. In addition, a QA-PAH spike was included in all GC–MS test batches. The obtained data was integrated using Environmental ChemStation (MSD ChemStation F.01.03.2357, Agilent Technologies, Inc. CA, USA) for target *n*-alkanes (*n*C10-*n*C36), and MassHunter Quantitative Analysis for GCMS (B.08.00, Agilent Technologies, Inc.) for target PAHs. The concentration of target components in each sample was normalized against the concentration of 30ab hopane. This recalcitrant compound is often used in biodegradation studies to correct for abiotic losses (Prince et al., 1994). Normalized degradation ratios are reported as percent of the normalized ratio in samples at day 0 of the experiment.

Biotransformation rates were calculated by non-linear regression analyses, using the “plateau followed by one-phase decay” option in GraphPad Prism 7.02 (GraphPad Software Inc., La Jolla, CA, USA). The X0 is equivalent to the lag-period, followed by a one-phase decay curve described by the following equation: $y = C_0 \cdot e^{-k1/t}$, where y is the concentration at time *t* of sampling, C_0 is the concentration at day 0 of incubation, and k_1 is the first-order rate coefficient. Half-life was determined as $\ln 2/k_1$. The plateau is the y value at infinite time and is set to 0 as it is assumed that all oil components are degraded given infinite time.

2.5. Microbial enumeration

Enumeration of total cell concentrations were performed by epifluorescence microscopy (1250 times magnification) of samples stained with the nucleic acid stain 4′,6-diamidino-2-phenylindol (DAPI) (Porter and Feig, 1980). The Most probable number (MPN) of viable heterotrophic (HM) and oil degrading (ODM) microorganisms were determined in 24-well sterile culture well plates as described by Brown and Braddock (1990). MPN of HM were determined in Difco Marine Broth 2216 (Becton, Dickinson and Company, NJ, USA), while ODM were determined in Difco Bushnell-Haas Broth (Becton, Dickinson and Company) supplemented with 30 g/l NaCl and 0.1% (vol/vol) of 250 °C + Troll B oil. The plates were incubated at 4 °C for a total of 14 and 35 days for HM and ODM, respectively, and checked for positive wells after day 3, 7, 14, 21, 28 and 35.

2.6. Microbial community analysis

DNA was extracted from filters using a modified method as presented in (Hazen et al., 2010), combined with the DNeasy PowerSoil kit (Quiagen GmbH, Hilden, Germany). Negative control samples, clean 47 mm polyethersulphone membrane filters, were included in the DNA extraction. DNA quality was assessed using a NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific, MA, USA), and DNA yield was quantified using Qubit 3.0 with dsDNA High Sensitivity kit (ThermoFisher Scientific). Characterization of microbial communities was carried out by amplicon sequencing of the 16S rRNA gene. Amplicons comprising the V3 and V4 region of the 16S rRNA gene were generated using the primers Ill338F (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNCCTACGGGWGGCAGCAG-3′) and Ill805R (5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGNNNNGACTACNVGGGTATCTAAKCC-3′) (Sigma Life Science, TX, USA). The PCR reactions were run for 33 cycles (98 °C 15 s, 55 °C 20 s, 72 °C 20 s) with 0.3 μM of each primer, 0.25 mM of each dNTP, 2 mM $MgCl_2$, Phusion Hot Start II High-Fidelity DNA Polymerase and Phusion buffer HF (ThermoFisher Scientific) in a total volume of 25 μL. To achieve equal concentrations of the amplicon for each sample, products were normalized using the SequalPrep™ Normalization Plate Kit (Invitrogen, Thermo Fisher). A second PCR was performed to attach index sequences to the normalized amplicons by using the Nextera XT Index Kit (Illumina, Inc., CA, USA). The reactions were run for 8 cycles (98 °C 15 s, 50 °C 20 s, 72 °C 20 s) with 2.5 μl of each index primer in a total volume of 25 μL. The other reaction conditions were as described for the first PCR. The indexed PCR products were normalized as described above, pooled, and concentrated by using Amicon Ultra-0.5 Centrifugal Filter Devices (Merck KGaA, Darmstadt, Germany). The concentrated sample was sequenced on a MiSeq lane with V3 reagents employing 300 bp paired end reads (illumina, Inc.), at the Norwegian Sequencing Centre (Oslo, Norway).

Sequence data was analyzed using AmpliconTagger, the National Research Council Canada's amplicon pipeline (Tremblay and Yergeau, 2019). Briefly, raw reads were scanned for sequencing adapters and PhiX spike-in sequences and remaining reads were merged using their common overlapping part with FLASH (Magoc and Salzberg, 2011). Merged sequences were filtered based on quality (Phred) score and remaining sequences were clustered at 100% identity and then processed for generating Amplicon Sequence Variants (ASVs) (Deblur v1.0.4) (Amir et al., 2017). ASVs having abundances < 25 were discarded, excluding 14,000 of 517,687 ASV sequences. Chimeras were removed with UCHIME reference (Edgar et al., 2011). A global read count summary is provided in Table S1, Supplementary Information. ASVs were assigned a taxonomic lineage with the RDP classifier (Wang et al., 2007) using an in-house training set containing the complete Silva release 128 database (Quast et al., 2013) supplemented with eukaryotic sequences from the Silva database and a customized set of mitochondria, plasmid and bacterial 16S sequences. The RDP classifier gave a score (0 to 1) to each taxonomic depth of each ASV. Each taxonomic depth having a score ≥ 0.5 was kept to reconstruct the final lineage. ASV37 classified as *Propionibacterium* was removed from the dataset. This specific bacterium is known as a typical contaminant introduced during DNA extraction and DNA clean-up (Sheik et al., 2018)

and was highly abundant in 3 of 4 indexed negative control samples and PCR blanks that were included in the amplicon library for sequencing. Taxonomic lineages were combined with the cluster abundance matrix obtained above to generate a raw ASV table, from which a bacterial organism ASV table was generated. Five hundred 1000 read rarefactions were then performed on this latter ASV table and the average number of reads of each ASV of each sample was computed to obtain a consensus rarefied ASV table. A multiple sequence alignment was obtained by aligning ASV sequences on a Greengenes core reference alignment (DeSantis et al., 2006) using the PyNAST v1.2.2 aligner (Caporaso et al., 2010). Alignments were filtered to keep only the hypervariable region of the alignment. A phylogenetic tree was built from that alignment with FastTree v2.1.10 (Price et al., 2010). Alpha (Richness, Shannon and Simpson index) and beta (Bray Curtis and weighted UniFrac distances) diversity metrics and taxonomic summaries were then computed using the QIIME v1.9.1 software suite (Caporaso et al., 2010; Kuczynski et al., 2011) using the consensus rarefied ASV table and phylogenetic tree (i.e., for UniFrac distance matrix generation).

2.7. Statistical analysis

Statistical analysis of degradation of hydrocarbons was performed using built in functions in GraphPad Prism 7.02. 2way ANOVA with Bonferroni's multiple comparison test was used to determine if the loss of hydrocarbons was significant between sampling days, and a two-tailed *t*-test was used to determine if degradation rates and lag-times were different between treatments.

All statistical analyses of microbial communities were performed using R-studio v. 1.1.463 (RStudio Team, 2015) and R software version 3.5.3 (R Core Team, 2019). Community structure was compared between samples by calculating Bray-Curtis dissimilarity, using the vegdist function available from the vegan (2.5–4) package (Oksanen et al., 2019). Analysis of differences between groups in the distance matrix were performed by PERMANOVA, using the adonis function in vegan. Pairwise comparisons between different treatments, were done by pairwise permutation MANOVAs analysis (pairwise.perm.manova) from the RVAideMemoire (0.9–72) package (Hervé, 2019). Similarity percentage (Simper) analysis was performed in vegan to determine which ASVs were contributing most to the Bray-Curtis differences between groups.

2.8. Other analyses

Dissolved oxygen (DO) was measured in the seawater phase of all samples. Aliquots were carefully poured into 100 ml glass flasks, to avoid mixing in excess oxygen. DO was determined using a DO meter connected to a BOD probe (YSI, Yellow Springs, OH, USA). Salinity was determined using a handheld S/Mill-E refractometer (ATAGO CO., LTD., Tokyo, Japan), measuring in the scale of 0–100 per-mill (‰).

3. Results

3.1. Physical parameters

The experiments with frazil ice required careful temperature control to avoid complete freezing of the SW or melting of the ice. The average air temperature in the climate room was −2.2 °C during the experimental period, with temperature peaks at approx. 2.6 °C caused by the defrosting cycle of the cooling system in the room (Fig. S2 A, Supplementary Information). The water temperature fluctuated between −2.6 and −1.0 °C, with an average of −1.9 °C in flasks without ice (Fig. S2 B, Supplementary Information). DO was measured in the water phase after each sampling and remained stable during the experimental period (Fig. S3, Supplementary Information), with an average of 10.4 ± 0.5 mg/L at the end of the experiment. DO was never measured below 8 mg/L. The salinity of the initial seawater was 35 ± 1.1‰. Desalination of the ice fractions in samples with added frazil ice (FI + oil and FI) resulted in increasing salinity of the seawater fractions throughout the experiment, reaching a maximum of 46 ± 1.1‰ at day 88 (Fig. S4, Supplementary Information). At the end of the experiment the seawater salinity was 40 ± 1.8‰, a reduction most likely caused by out-crystallization of salt. Salt crystals were observed in the screw treads of the bottles and the cap. The salinity of the frazil ice fraction did not change throughout the experiment, ranging between 4 and 5‰ (average 4.8 ± 0.4‰). The seawater without ice (SW + oil and abioticSW + oil) had a relatively constant salinity between 34 ± 0.5‰ and 38 ± 2.3‰ (Fig. S4, Supplementary Information).

3.2. Oil biodegradation

GC-FID analyses did not show significant depletion of TPH, neither in the FI + oil samples (pooled results for seawater and frazil ice fraction), nor in SW + oil samples (Fig. S5 and S6, Supplementary Information). However, depletion of *n*-alkanes (Σ*n*C14-*n*C32 alkanes) and PAHs were measured, both in FI + oil and SW + oil treatments. While *n*-alkane depletion at the end of the experiment was 83.3 ± 6.0% and 79.2 ± 9.5% in FI + oil and SW + oil, respectively, the corresponding PAH depletion was 46.6 ± 9.0 and 77.6 ± 0.8%. The degradation of oil components therefore progressed faster in samples added seawater and oil (SW + oil) than in samples treated with frazil ice, seawater and oil (FI + oil), because of the greater loss of PAHs. The depletion of these oil compound groups was mainly caused by biodegradation, since losses in sterilized controls (abioticSW + oil) were small or insignificant (Fig. S6, Supplementary Information).

3.3. Biotransformation of targeted oil compounds and groups

Depletion of single targeted oil compounds and compound groups was determined after normalization against the recalcitrant biomarker 30ab hopane. These included *n*-alkanes from *n*C14 to *n*C32, and the PAHs C0-C4 naphthalene, C0-C4 fluorene, C0-C4 phenanthrene, C0-C4 dibenzothiophenes, C0-C4 fluoranthene and C0-C4 chrysenes. No loss of 30ab hopane was measured during the experiment (results not shown).

Biotransformation of single *n*-alkanes in both treatments containing oil (FI + oil and SW + oil), were insignificant until 55 days of incubation. The amounts of < *n*C25 were then significantly decreased compared to day 0 ($p < 0.05$ for FI + oil and $p < 0.001$ for SW + oil samples), as shown in Fig. 1. The *n*-alkane degradation patterns in FI + oil and SW + oil was comparable (Fig. 1), while the isoprenoids pristane and phytane were degraded faster in samples containing frazil ice (FI + oil) than in samples only treated with oil dispersion (SW + oil). The delayed degradation of pristane and phytane, observed in both treatments, is typical for biodegradation of branched alkanes (Douglas et al., 1996; Oudot et al., 1998; Oh et al., 2001). First-order degradation kinetics of the sum of *n*-alkanes in the two treatments are shown in Table 2. After estimated lag times of 45 (FI + oil) and 34 (SW + oil) days, the half-lives of *n*C14 to *n*C32 were 17 days (62 days including lag-time) for FI + oil and 39 days (73 days incl. Lag-time) for SW + oil. Biotransformation of total *n*-alkanes was slightly faster in samples treated with oil and frazil ice than in samples with added oil but no ice, where both lag-time and degradation rate were significantly different from each other ($p < 0.001$).

Compared to day 0, PAHs degradation was insignificant before day 55 of incubation ($p < 0.001$ for FI + oil and $p < 0.05$ for SW + oil), similar to the *n*-alkane measurements. Parent PAHs were degraded faster than the alkylated homologues, following a pattern where the degradation decreased with increasing alkyl substitution (Fig. 2). Biotransformation of PAHs was also related to ring-number, and degradation of larger PAHs was only observed in SW + oil, where degradation of fluoranthene and pyrene by the end of the experiment

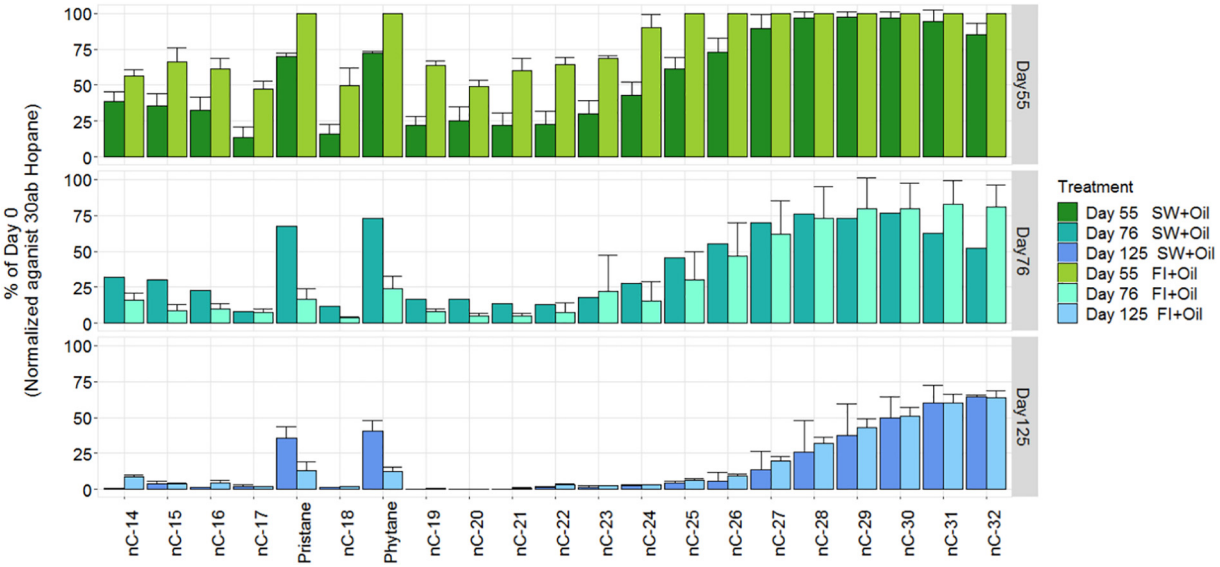

Fig. 1. Degradation of target n-alkanes in dispersed oil with frazil ice (FI + oil), and dispersed oil w/o frazil ice (SW + oil). The losses are shown as percentages of day 0, after normalization against 30ab Hopane. Different colours represent the sampling days from day 0 to day 125.

were significant compared to day 0 ($p < 0.001$ for both substrates). The biotransformation kinetics of PAHs differed more between the two treatments than degradation of *n*-alkanes, as shown in Table 2. The half-life (incl. lag-time) of the sum of target PAHs was almost three times longer in samples with frazil ice and oil (FI + oil; 189 days) than in samples with just oil (SW + oil; 67 days). The half-lives (incl. lag-time) of naphthalenes and 2- to 3-ring PAHs were 115 and 138 days in FI + oil, and 52 and 76 days in SW + oil, respectively. Degradation rates were significantly faster ($p < 0.001$) for both naphthalenes and 2–3-ring PAHs, as well as for the sum of PAHs, in seawater with oil (SW + oil) compared to frazil ice and oil (FI + oil). Degradation kinetics could not be calculated for 4–6 ring PAHs because of the limited loss of these compounds.

3.4. Biomass concentrations

The total number of microbial cells as determined by epi-fluorescence microscopy, increased throughout the experiment. The cell numbers increased by factors of 5 to 10 in the seawater phases (SW + oil and FI + oil/SW) during the experimental period, from 1×10^4 cells/ml for all treatments to an average of 5.6×10^5 cells/ml for FI + oil samples, and 5.7×10^5 for SW + oil samples (Fig. S7, Supplementary Information). In the FI + oil/ice fraction of the FI + oil samples the cell numbers increased even more, by a factor of > 100, from initial concentrations of 5.7×10^3 cells/ml to 6.0×10^5 cells/ml at the end of the experiment. The cell numbers in the oil-containing samples therefore increased more than in the biotic control without oil (FI, containing only seawater and frazil ice), and which had 2.3×10^5 cells/ml at the end of the experiment.

3.5. Microbial population structures and community succession

The microbial community structure changed over time and was shaped both by the addition of oil and addition of frazil ice. After 30 days of incubation, microbial communities had changed from an initially diverse community, to a community where 20 genera made up 84–99% of the total relative abundance (Fig. 3) in the oil treated samples (FI + oil and SW + oil), with most of the taxa belonging to the class Gammaproteobacteria. Variations at the family level are shown in Fig. S8, in the Supplementary Information. The microbial communities in the biotic controls (FI) without oil did not undergo the same change as the oil treatments and did not change much after the initial loss of diversity. The most abundant taxa throughout the experiment in the biotic control included *Sulfitobacter, Colwellia, Zhongshania, Pseudohoglella* and Acidimicrobiales (Fig. 4 and Fig. S8).

All biological treatments (FI + oil, SW + oil and FI) experienced an initial loss of ASV richness, due to the bottling of the water (Fig. 4), from day 0 to day 30. The ASV richness decreased further with time to < 50 for treatments containing oil (FI + oil and SW + oil), but not in the biotic control without oil (FI), where the diversity remained

Table 2
Degradation kinetics for samples treated with dispersed oil with frazil ice (FI + oil) and dispersed oil without frazil ice (SW + oil). Lag-period (days), first-order rate coefficients (k1, days^{-1}), half-lives (days) and goodness-of-fit (R^2) is calculated from one-phase exponential decay.

Compound group	Ice/no ice	Lag period (days)	Rate coefficient (k1)	Half-life (days)[a]	Goodness of fit (R^2)
*n*C14-*n*C32 alk.	FI + oil	45.1	0.041 (± 0.009)	61.9	0.91
	SW + oil	34.4	0.018 (± 0.003)	73.1	0.91
Naphthalenes	FI + oil	35.0	0.009 (± 0.002)	114.9	0.74
	SW + oil	35.0	0.041(± 0.010)	51.8	0.90
2–3 ring PAHs	FI + oil	35.0	0.007 (± 0.002)	138.4	0.48
	SW + oil	25.4	0.014 (± 0.005)	75.7	0.72
4–6 ring PAHs	FI + oil	[b]	[b]	[b]	[b]
	SW + oil	[b]	[b]	[b]	[b]
Sum PAHs	FI + oil	35.0	0.004 (± 0.001)	189.4	0.51
	SW + oil	31.1	0.020 (± 0.004)	66.6	0.87

[a] Half-life determined as the sum of lag-period and half-life calculated from the rate coefficient.
[b] Degradation rates could not be estimated (k1 < 0.001).

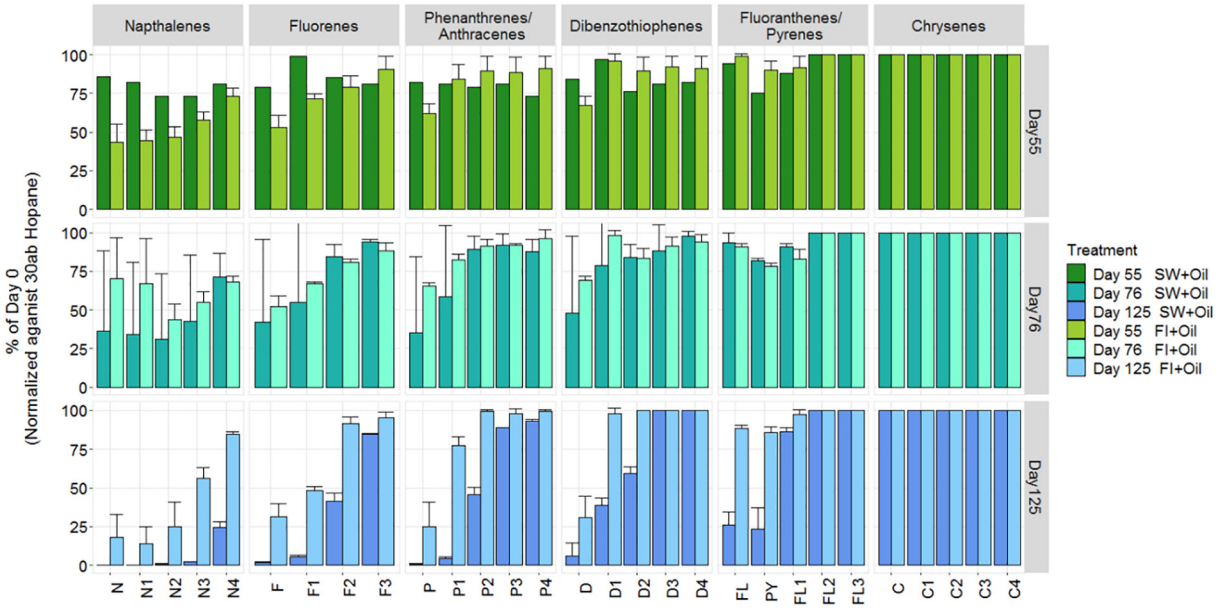

Fig. 2. Degradation of target PAHs in seawater with frazil ice (FI + oil), and without frazil ice (SW + oil). The losses are given as percentages of day 0, after normalization against 30ab Hopane. Different colours represent the sampling days from day 0 to day 125.

constant after day 30 (Fig. 4A). FI samples (biotic controls without oil) had a higher Shannon's diversity than the oil treated samples. The ice fraction from the FI + oil treatment had lower ASV richness and Shannon's diversity than the SW fractions from the same samples, while the SW + oil samples had a higher diversity than in the sea water fraction of the FI + oil samples (Fig. 4B). After 55 days of incubation one ASV (ASV 1) became dominant in samples from the FI + oil treatment, first in the ice fraction (day 55; FI + oil/ice) and later in the seawater fraction (day 76; FI + oil/SW). This ASV was assigned to the genus *Perlucidibaca* (family *Moraxcellaceae*). ASV1 continued to predominate the FI + oil treated samples (both ice and seawater fractions) throughout the experiment (Fig. 3). SW + oil treated samples were dominated by *Cycloclasticus* (family *Piscirickettsiaceae*) at day 76, however, the abundance of this genus diminished as *Perlucidibaca* became increasingly abundant in these samples towards the end of the experiment. Other genera were also present in samples with dispersed oil (FI + oil and SW + oil) throughout the experiment. *Alcanivorax, Colwellia, Sphingorhabdus, Sulfitobacter* and some unclassified genera belonging to the order Acidimicrobiales were present in equal but low abundance in SW + oil treated samples. In FI + oil treated samples *Sulfitobacter* (family *Rhodobacteraceae*) became relatively abundant in the seawater fraction after 76 days of incubation. *Colwellia, Sphingorhabdus* and other Acidimicrobiales were also present, but in lower relative abundance.

At the end of the experiment (day 125), *Cycloclasticus* also emerged in the FI + oil samples, making the microbial communities in the two oil treatments more similar. SIMPER analysis, of the total ASV table, comparing the two oil treatments (FI + oil and SW + oil) at each sampling day (Table S2, Supplementary Information), showed that differences early in the experiment (day 30) were caused by three ASVs (ASV452, 7 and 3). ASV452 (classified as the genus *Sulfurimonas*), was contributing the most to the average dissimilarity (28%) between the FI + oil and SW + oil, being highly present in the SW + oil samples. After day 55, the dissimilarity between treatments was caused by the high abundance of ASV1 (*Perlucidibaca;* 34%) in FI + oil samples and ASV240 (*Cycloclasticus;* 11%), which was more abundant in SW + oil. At day 76, abundances of ASV1 were 0.76 and 0.10, and ASV240 0.00 and 0.66 for the FI + oil and SW + oil treatment respectively, contributing to 40% (ASV1) and 39% (ASV240) to the average dissimilarity. However, the abundance of these ASVs became more similar for the FI + oil and SW + oil treatment with time. Relative abundance at day 125 of ASV1 was 0.69 and 0.52, and 0.12 and 0.18 for ASV240 in FI + oil and SW + oil, respectively.

Succession within the microbial communities was further examined by PCoA ordination based on Bray-Curtis dissimilarities (Fig. 5). Patterns along PCoA2 showed the effect of treatment on the microbial community dynamics. Samples from the biotic control (FI) formed a separate cluster after day 30 and were clearly different from the ordination of the FI + oil and SW + oil treatments. The shifts in microbial community structure of the FI + oil (both ice and seawater fractions) and SW + oil samples were similar to one another, however, following two different paths along PCoA 2, dependent on treatment. After day 75, samples from the FI + oil treatment clustered together. Over time, samples from the SW + oil treatment shifted along PCoA 1, clustering together with the FI + oil samples, showing a clear succession towards a more similar community by day 125.

The observed changes in the microbial community were shaped by time, presence or absence of frazil ice and the presence or absence of dispersed oil (Table S3, Supplementary Information). PERMANOVA analysis of the distance matrix, showed that sampling day was identified as the variable with the most influence on the microbial community, explaining 42% of the variation among ASVs. SIMPER analysis comparing differences between sampling days for each treatment showed that one ASV classified as a *Perlucidibaca* (ASV1) accounted for most of the variance between sampling days in both ice and SW fractions of the FI + oil treatment (Table S4, Supplementary Information), whereas ASV240 (*Cycloclasticus*) accounted for most of the time related differences within the SW + oil treatment. However, at the end of the experiment an increase of the relative abundance of ASV240 (0.0 on day 88 and 0.18 on day 125) in the sea water fraction of the FI + oil treatment contributed most to the observed dissimilarity. The opposite was the case in the SW + oil treatment, where an increase in the abundance of ASV1 (0.18 on day 88 and 0.52 on day 125) contributed most to the dissimilarity within in the microbial community.

In summary, microbial communities in the two oil treatments changed over time, and the presence of ice appeared to affect the community composition in the seawater, as the communities in FI + oil samples differed from those in the SW + oil samples. The differences in seawater communities between oil with and without frazil ice (FI + oil and SW + oil) from the mid to late part of the experiment could be explained by the presence/absence of *Perlucidibaca* and *Cycloclasticus*,

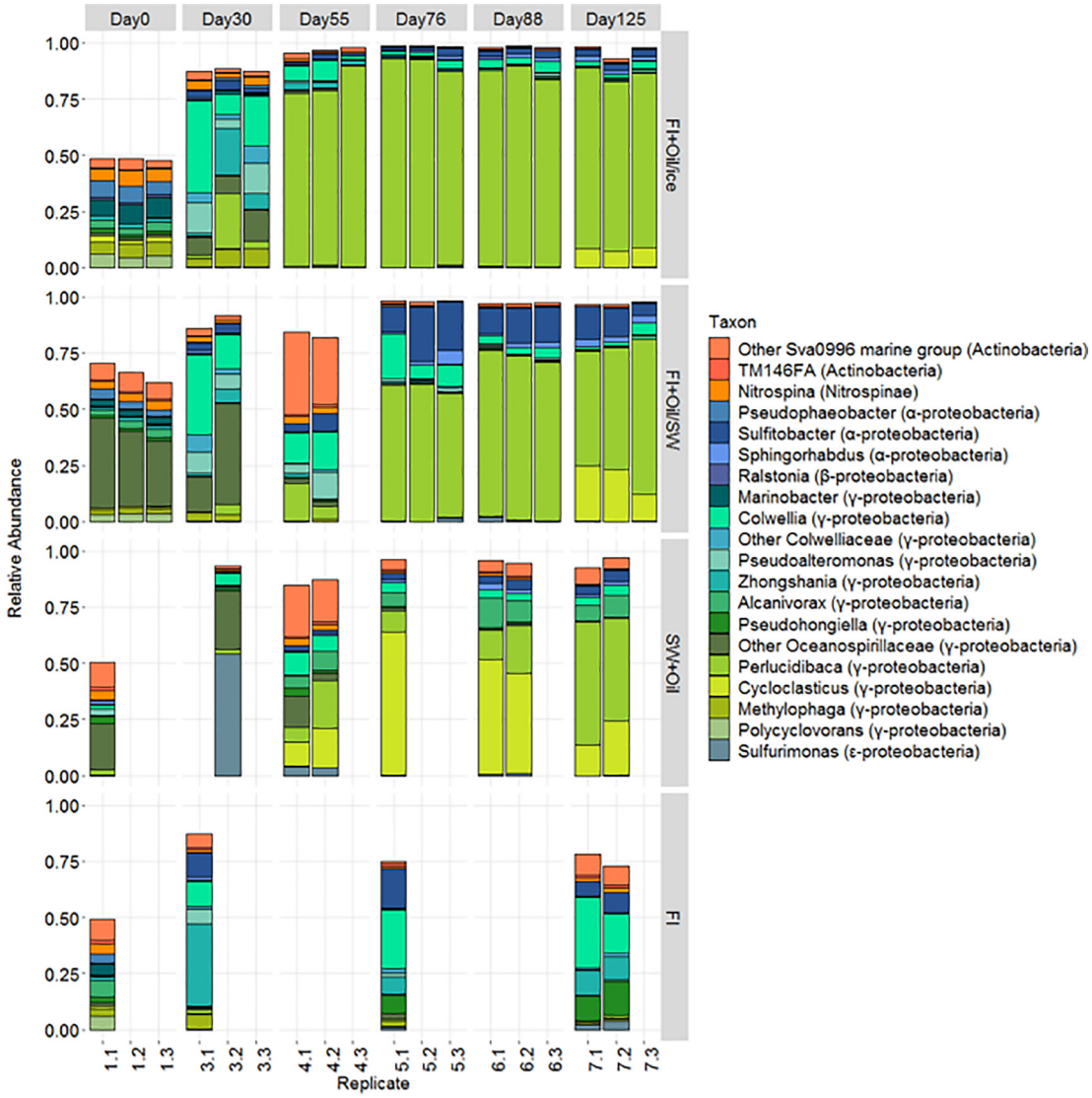

Fig. 3. Relative abundance of the 20 most abundant bacterial families during the experimental period of 125 days. The different treatments are shown on the right of each panel: dispersed oil with frazil ice (FI + oil/SW and FI + oil/ice); dispersed oil w/o frazil ice (SW + oil); and seawater without oil with frazil ice (FI). Replicate samples at each sampling day (0, 35, 55 76, 88 and 125) are shown on the lower horizontal axis.

with dominance of *Perlucidibaca* and *Cycloclasticus* in FI + oil and SW + oil, respectively. However, at the end of the experiment (day 125), the two treatments became more similar, with *Cycloclasticus* emerging in frazil ice (FI + oil) samples and increasing predominance of *Perlucidibaca* in oil without ice (SW + oil).

4. Discussion

The efficiency of oil biodegradation is dependent on oil surface-to-volume ratio, where small oil droplets are degraded faster than larger ones (Brakstad et al., 2015a). The ability to generate dispersions may therefore be regarded as crucial for biodegradation of oil, especially in ice-infested areas with reduced wave activity (Weber, 1987). For oil spills near ice-infested waters or the ice edge, the use of dispersants has been suggested for oil spill response when the oil is still in open water (Lewis and Prince, 2018). In this study, weathered oil was therefore premixed with a dispersant to generate oil-dispersions at a low concentration that is considered environmentally relevant (Lee et al., 2013). The dispersions were mixed with frazil ice and seawater to simulate a scenario where oil comes in contact with newly formed ice. A control experiment was also performed simultaneously with dispersed oil in seawater without ice, to compare the impacts of the ice on oil biodegradation.

Negligible loss of bulk oil was measured in both the oil treatments with and without frazil ice (FI + oil and SW + oil). Even though losses of *n*-alkanes were evident, the fraction of *n*-alkanes and PAHs only constitute about 5% of the total oil used in these experiments (the dispersible naphthenic Troll B oil). As determined in previous biodegradation studies with fresh Troll oil, low to negligible degradation of TPH was observed at low seawater temperatures (Brakstad et al., 2018). The Troll oil is considered to be rich in the recalcitrant unresolved complex mixture (UCM) (Melbye et al., 2009), and a large fraction of UCM could therefore contribute to the limited degradation of TPH. Another important factor could be the temperature, since oil degradation has been shown to be significantly decreased at seawater temperatures lower than 5 °C (Lofthus et al., 2018). At low seawater temperature, oils become more viscous, partly affected by the content of asphaltenes and wax (Bowman et al., 1997; Faksness et al., 2015), which results in decreased biodegradability (Haus et al., 2001).

So far, no experiments have shown extensive degradation of oil frozen into sea ice. Exposure to oil has stimulated the growth of potential oil degraders in sea ice, but with no detectable degradation of

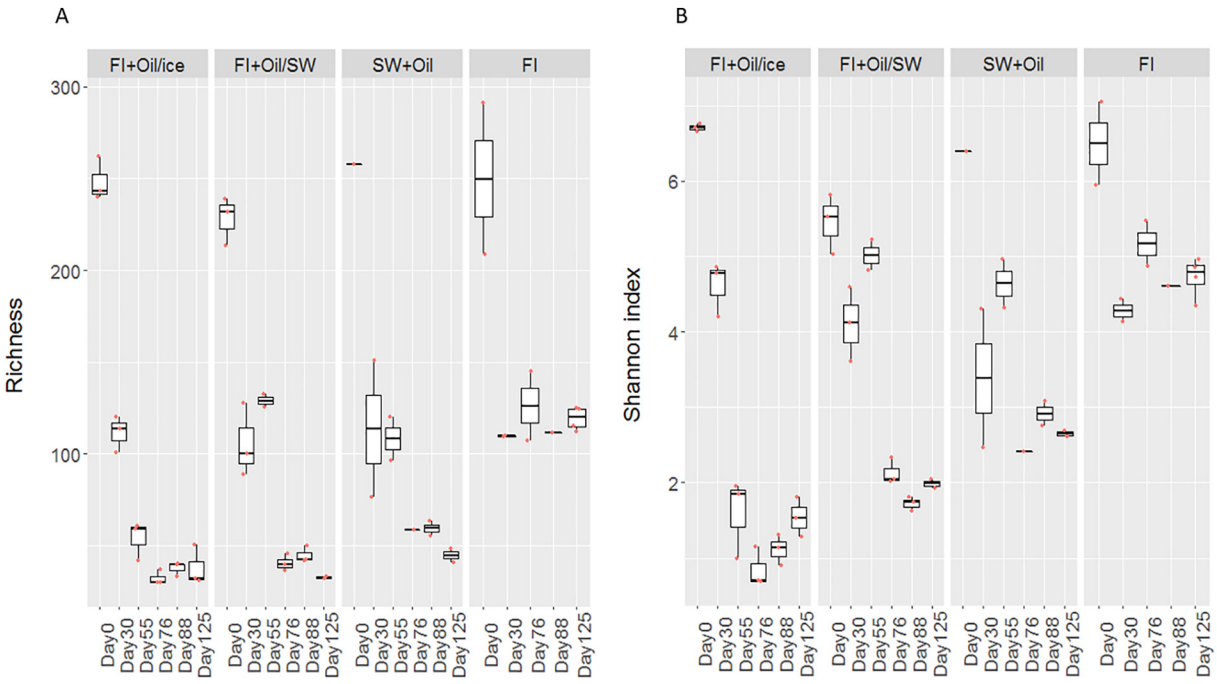

Fig. 4. Changes in species richness (A) and in the Shannon index diversity (B) during the 125 days of incubation, in the different treatments; dispersed oil with frazil ice (FI + oil/SW and FI + oil/ice); dispersed oil w/o frazil ice (SW + oil); and seawater without oil with frazil ice (FI).

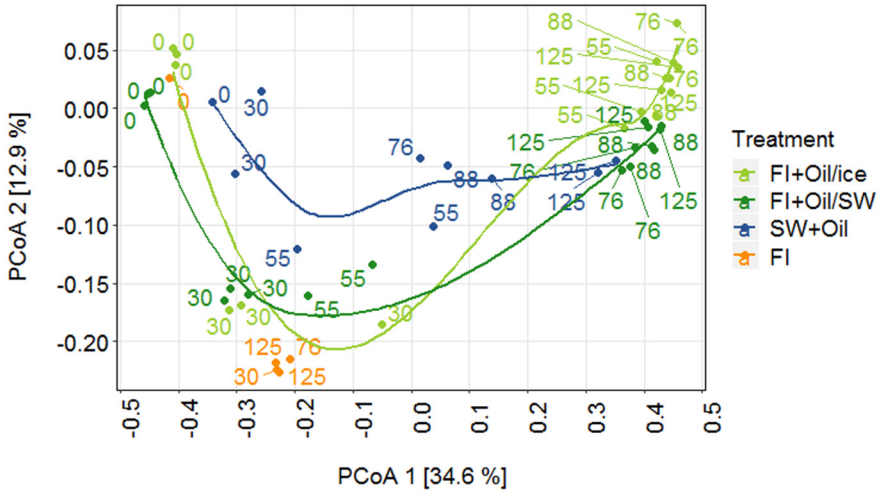

Fig. 5. Change in microbial community structure over time based on Bray-Curtis similarity. Principal component (PCoA) 1 explains 34.6% of the variance between samples, whereas PCoA 2 accounts for 12.9%.

the total oil (Gerdes et al., 2005; Brakstad et al., 2008; Boccadoro et al., 2018; Vergeynst et al., 2019). However, loss of oil components has been observed in the bottom sections of ice, being in contact with the underlying seawater, proposed to be caused both by biodegradation and dissolution (Boccadoro et al., 2018; Vergeynst et al., 2019; Faksness and Brandvik, 2008). This scenario could be comparable to the FI + oil treatment in this experiment, where ice is in contact with seawater throughout the experiment.

In both experiments with dispersed oil in the presence and absence of frazil ice, target compounds or compound groups were degraded according to their size and complexity. Alkanes were biotransformed at a rate inversely proportional to chain length and branching, while PAH losses were reduced with increased number of aromatic rings and degree of alkylation, a common pattern for biodegradation of oil in seawater (Brakstad et al., 2018; Scheibye et al., 2017; McFarlin et al., 2014; Venosa and Holder, 2007).

Degradation patterns of *n*-alkanes were comparable in both oil treatments (FI + oil and SW + oil) after the 125 days of incubation, whereas degradation of naphthalenes and PAHs were significantly slower in the presence than in the absence of frazil ice, with longer lag-times and lower rate coefficients in the ice treatment (FI + oil). Although both *n*-alkane and PAH degradation were observed, degradation was slow compared to other relevant studies for both treatments, with long lag-times of *n*-alkanes and PAHs, as well as low degradation rates for the PAHs, particularly in ice treated samples. Vergeynst et al. (2019) observed complete removal of *n*C10- *n*C27 within 31 days of exposure to sub-ice seawater. However, depletion of PAHs was not caused by biodegradation in their studies. Almost 60% of *n*-alkanes and a substantial amount of PAHs (including 20% loss of the four-ringed PAH chrysene) were biodegraded when incubated as dispersed oil in Arctic SW at -1 °C, over a period of 63 days (McFarlin et al., 2014), whereas Garneau et al. (2016) reported complete removal

of *n*-alkanes and PAHs within 11 days at −1.7 °C, with sub-ice seawater as inoculum. However, long lag phases and limited degradation of total oil and PAHs have been observed previously in pristine seawater from Disko Bay in Greenland (Brakstad et al., 2018; Scheibye et al., 2017), both suggesting that pre-adaption to oil and sufficient nutrients is crucial for efficient degradation. Nevertheless, it is important to emphasize that direct comparison of degradation rates between studies is challenging, because of the variety of factors affecting the degradation process, as well as the use of different experimental systems and strategies.

The slow degradation observed in our studies may have been affected by the hydrocarbon composition of the weathered oil. During weathering at 250 °C, significant amounts of the most water- soluble and easily degradable compounds are lost by evaporation. In addition, reduced bioavailability at low temperature may have slowed the onset of degradation. Degradation of longer-chain *n*-alkanes (≥nC25) and larger PAHs (≥ 3 rings) are typically suppressed at temperatures close to 0 °C, compared to more water-soluble hydrocarbons (Vergeynst et al., 2018; Lofthus et al., 2018). In this study, the temperature was kept at approx. -2 °C, colder than other experiments studying biodegradation of dispersed oil in seawater, and we expect that the effects of the temperature on the availability and uptake of substrate are limiting the biodegradation capacity of the microbial community.

While hydrocarbon chemistry and bioavailability may be the major cause of slow degradation, the difference in degradation of target compounds between treatments could be a result of differences between the microbial communities in the presence/absence of frazil ice (discussed more later in this section). Vergeynst et al. (2018) showed that temperature influenced the microbial community structure at a given incubation time, and that low temperature would delay microbial community succession, but that the communities were similar at the same state of substrate consumption. Parallels can be drawn to our study where the onset of PAH degradation in samples containing frazil ice was particularly delayed compared to samples without ice, but at the end of the experiment the microbial communities in both treatments (FI + oil and SW + oil) became more similar, also reflected by gradually more similar hydrocarbon composition. This is off course a two-way process, where oil-degrading microbes will shape the state of substrate composition, and the available substrate will determine the succession of the microbial community.

While pristine Arctic seawater typically shows a bacterial distribution between Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Bacteroidetes (Flavobacteriia) (Kirchman et al., 2010; Hatam et al., 2014; McFarlin et al., 2017), microbial communities in sea ice have a predominance of Gammaproteobacteria and Bacteroidetes (Flavobacteriia) (Brakstad et al., 2008; Hatam et al., 2014; Boetius et al., 2015; Boccadoro et al., 2018). When crude oil enters the marine environment, the microbial communities usually undergo a rapid shift towards dominance of Gammaproteobacteria (Yakimov et al., 2004), and members of this class were already predominant at the first sampling (day30) in both oil treatments with or without frazil ice (FI + oil and SW + oil). Both treatments (FI + oil and SW + oil) were completely dominated by only a few taxa. The diversity (Shannon index) was generally higher in the controls without oil (FI), than in the oil samples throughout the experiment, thus oil appeared to be a more important driver of low diversity than low temperatures or the presence of ice.

Generally, initial low diversity is followed by diversification of the microbial communities, in response to degradation of increasingly complex substrates (Brakstad et al., 2004; Lofthus et al., 2018; Ribicic et al., 2018c; Vergeynst et al., 2018; Garneau et al., 2016). However, in this study we did not see the typical increased diversity of the microbial communities over time, and relatively few taxa were present after 125 days of incubation. This indicates that the degradation process was still in its early phase at the end of the experiment, as a result of the slow degradation process at the very low incubation temperature. In most other studies with cold seawater, community diversification (and loss of hydrocarbons) occurred earlier in the experimental period, most probably as a result of the higher seawater temperatures of 0 to 5 °C (Brakstad et al., 2018; Ribicic et al., 2018c). Results from other studies have also shown that temperature had an effect on the microbial community structure at a given incubation time (Ribicic et al., 2018a; Lofthus et al., 2018), and that low temperature would cause a delayed microbial community succession, but that the communities were similar at the same state of substrate consumption (Vergeynst et al., 2018).

The changes in the microbial communities during the experiment were associated with increasing abundance of previously identified hydrocarbon degraders, both in the FI + oil and SW + oil treatments. *Colwellia* was the most prominent taxon in the early phase of the experiment (day 30) in FI + oil samples. *Colwellia* was also abundant in the biotic control (FI) throughout the experiment. *Colwellia* are known psychrophiles (Methé et al., 2005) and are generally abundant in cold seawater. Increased abundance of *Colwellia* could therefore be the result of cold temperature as well as oil contamination (Ribicic et al., 2018c; Krolicka et al., 2017). *Colwellia* has been linked to the degradation of several types of hydrocarbons from simple aromatic (Redmond and Valentine, 2012; Hu et al., 2017) to PAHs in Arctic deep sea sediments (Dong et al., 2015). Members of this genus have previously been associated with oil degradation both in cold seawater (Brakstad et al., 2018; Brakstad et al., 2008; Garneau et al., 2016; Vergeynst et al., 2019) and marine ice (Brakstad et al., 2008; Boccadoro et al., 2018).

Other typical oil degraders were not abundant in any of the oil treatments (FI + oil and SW + oil) until late in the experiment (after day 76), including *Sulfitobacter* in samples with frazil ice (FI + oil), and *Alcanivorax* and *Cycloclasticus* in SW + oil samples. *Sulfitobacter* and other unassigned *Rhodobacteraceae* are suspected to be involved in the degradation of more recalcitrant compounds (Kostka et al., 2011; Ribicic et al., 2018a), and typically increase in abundance later in the degradation process (Brakstad et al., 2018, Ribicic et al., 2018c). *Alcanivorax* is regarded as an alkane degrader, usually abundant early in oil spill events (Garneau et al., 2016; Head et al., 2006). Interestingly, *Alcanivorax* increased in abundance only later in the experiment. *Oceanospirillaceae* were abundant early in the study (days 0–55), in both treatments with and without frazil ice. This period was associated with degradation of shorter *n*-alkanes. Members of *Oceanospirillaceae* are typically associated with depletion of *n*-alkanes, and these microbes are usually abundant mainly in the early part of the oil degradation periods, with a subsequent decline in abundance (Dubinsky et al., 2013; Ribicic et al., 2018a). Taxa typically associated with cold water were also identified in samples with oil, like the Sva0996 marine group, previously identified in Antarctic water (Richert et al., 2015). However, this group has previously not been associated with hydrocarbon degradation.

The most obvious difference in the microbial communities between the oil treatments with and without frazil ice (FI + oil and SW + oil), was the emergence and succession of the two genera *Perlucidibaca* (*Moraxellaceae*) and *Cycloclasticus* (*Piscirickettsiaceae*). After day 76, *Cycloclasticus* was predominant in SW + oil treated samples. Members of *Cycloclasticus* are considered to be obligate hydrocarbonoclastic bacteria (Yakimov et al., 2007), associated with PAH degradation (Wang et al., 2008; Dyksterhouse et al., 1995). *Cycloclasticus* has been identified as a PAH degrader in both Arctic and temperate seawater (Ribicic et al., 2018c; Brakstad et al., 2015b; Vergeynst et al., 2018), and correlations between degradation of 2- to 3-ring PAHs and increased abundance of *Piscirickettsiaceae* have been determined during biodegradation studies of dispersed Troll oil at 5 °C (Ribicic et al., 2018a). *Cycloclasticus* did not emerge in oil samples with ice (FI + oil), before the last day of sampling, reflecting the slow PAHs degradation in the presence of ice. The FI + oil samples were completely dominated by an ASV affiliated to *Perlucidibaca* (ASV1). Members of *Perlucidibaca* have previously been identified as a part of psychrophilic hydrocarbon degrading communities in seawater (Vergeynst et al., 2018), but not in the

presence of ice (until our study). *Perlucidibaca* became predominant in the ice fraction of the FI + oil samples at day 55 of the experiment. In SW + oil samples, *Perlucidibaca* did not become dominant before the last day of sampling (day 125). The presence of ice therefore seemed to be a selective stressor, favouring the growth of *Perlucidibaca.* This genus also emerged earlier in the ice than the seawater fraction of FI + oil samples and persisted throughout the experiment. This further emphasized the role of ice as a selection parameter for these bacteria, and that *Perlucidibaca* might be associated directly with the frazil ice crystals. It must be emphasized that the presence of ice also resulted in increased salinity in the seawater. In sea ice the salinity of the surrounding water will increase due to salt rejection of the maturing ice, and the increased seawater salinity in the samples with frazil ice is thus a realistic feature of an icy environment. Increased salinity may therefore be a microbial selection criterium, since salinity is well-known to structure microbial communities (Herlemann et al., 2011; Ewert and Deming, 2014). However, the same ASVs were observed in the two treatments (with and without ice), both for *Cycloclasticus* and *Perlucidibaca*. We can surmise that the presence of frazil ice selected for but did not inhibit the growth of certain members of the oil degrading community.

Another member of *Moraxellaceae, Paraperlucidibaca,* has a 16S rDNA sequence similar to the ASV1 classified as *Perlucidibaca* in this experiment (Teixeira and Merquior, 2014). Sequence identities between all matches of *Perlucidibaca* and *Paraperlucidibaca* to ASV1 in the RDP database were > 94% identical to each other. Whereas few other studies have identified *Perlucidibaca* as an oil degrader (Vergeynst et al., 2018), *Paraperlucidibaca* have previously been classified as a part of an oil degrading community also in cold environments (Brakstad and Bonaunet, 2006; Lo Giudice et al., 2010). In addition, an isolated strain of *Paraperlucidibaca* was shown to degrade Tween 80 (Yoon et al., 2013). Tween is often used as the oleophilic part of dispersants and are structurally similar to saturated hydrocarbons. We therefore suspect that *Perlucidibaca* is mainly degrading *n*-alkanes, and possibly dispersant in samples treated with dispersed weathered oil, as psychrophilic microorganisms are known to be able to fully mineralize oil and dispersant at cold temperatures (McFarlin et al., 2014).

5. Conclusions

Biotransformation of targeted oil compounds and compound groups occurred at low temperature and in both the presence and absence of frazil ice, but at different rates. Total *n*-alkanes were degraded faster in the presence than the absence of the ice, whereas the opposite was the case for naphthalenes and 2–3 ring PAHs. No degradation of 4–6 ring PAHs was observed in any of the treatments.

Microbial communities changed over time as a result of active oil compound degradation. The microbial communities were shaped not only by time and the presence of oil, but also the presence or absence of frazil ice. Differences between the two treatments (with and without frazil ice) were caused by the high abundance of two ASVs, one classified as a *Perlucidibaca* in ice and the other as a *Cycloclasticus* in SW without ice. Differences in the microbial communities were possibly the explanation for the differences in degradation rates between treatments. While *Cycloclasticus* seemed to play a role in PAHs degradation, we suspected *Perlucidibaca* to be a more efficient *n*-alkane degrader.

However, the concentration of the total oil was not reduced to any significant degree, suggesting that with continued evolution of frazil ice into solid ice forms, we would expect oil to freeze into the ice matrix and most likely persist throughout the icy season.

CRediT authorship contribution statement

Synnøve Lofthus: Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing - original draft. **Ingrid Bakke**: Methodology, Resources, Writing - review & editing, Supervision. **Julien Tremblay**: Software, Formal analysis. **Charles W. Greer**: Resources, Writing - review & editing. **Odd Gunnar Brakstad**: Conceptualization, Methodology, Writing - review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2020.111090.

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